Phlebotomus (Larroussius) orientalis (Diptera: Psychodidae) as a probable secondary vector of visceral leishmaniasis in Kenya

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According to the World Health Organization's reports, leishmaniases in humans are caused by 20 species of protozoa, belonging to the genus Leishmani a^1 . The parasites are transmitted by the bite of a female tiny, 2-3 mm long insect vector, the phlebotomine sandfly¹. Leishmaniasis endemic areas in Kenya are-West Pokot, Kitui, Machakos, Meru, Koibatek and Kajiado districts^{2–6}. Kala-azar (Visceral leishmaniasis) in these endemic sites is transmitted by Phlebotomus martini (Diptera: Psychodidae)^{5,6}, which predominantly lives and breeds in termite mounds, while in Sudan the same disease is transmitted by P. orientalis which breeds in soil cracks⁷. Previous reports of the existence of P. orientalis in Kenya suggested that this species did not occur in large numbers to cause any transmission of visceral leishmaniasis^{4,8}. However, in 2000, 2003 and 2006 some kala-azar epidemics were reported in Wajir District Hospital, north-eastern Province and Makagalla location, Merti division, Isiolo district, Eastern Province, Kenya⁹. Phlebotomus orientalis is the only member of the Larroussius Nitzulescu group that has been associated with L. *donovani* transmission in the sub-Saharan Africa^{10,11}. It is for this reason the study was carried out to investigate its possible role in the transmission of kalaazar in an endemic area where P. martini, the known vector of the disease in other parts of Kenya was seemingly absent but transmission has been going on.

Merti Division in Isiolo district, Eastern Province, lies 420 km north-east of Nairobi, Kenya. The location of the town is 1.066488° N, 38.657372° E and 347 m asl. The neighbouring districts are— Samburu to the west, Marsabit to the north, Wajir to the east, Meru and Garissa to the south. This is a semi-arid area that is inhabited by the Boran tribe which is a pastoralist, and keeps goats, sheep, cattle, camels and donkeys. The vegetation of the study area is characterized by *Acacia seyal* trees, *Prosopis juliflora* (locally known as 'Mathenge') and *Balanites aegyptica* which are evergreen for a greater part of the year. In kala-azar endemic areas in Sudan, *P. orientalis* distribution is associated closely with *Acacia* and *Balanites* species¹².

The study area is a place where an epidemic of kalaazar had been reported⁹. During the study, sandflies were trapped from two selected villages, namely Biliqi and Ollagana using SSAM light-traps (John W. Hock, Co; Gainsville, FL, U.S.A.) at night during 24-26 January 2009. These traps were positioned on Acacia trees next to ground cracks where P. orientalis is thought to breed and/or rest¹². On the following morning, the sandflies were aspirated from the collection nets and the live ones put in a large feeding cage where they were given some apple slices to sustain them. The dead sandflies were kept in vials for transportation to the laboratories in Nairobi for dissection and mounting. All the sandflies were dissected just to show the identification features only but not parasites. They were mounted in chloral hydrate solution and identified using standard taxonomic keys¹³.

In all the trapping sites, GIS points were taken for clarity and future reference. Records regarding normalized difference vegetation index (NDVI) were also taken. At the trapping sites, geographical coordinates were recorded using GPS hand-held receiver, Etrex GARMIN. Details of the study sites and environmental conditions that were recorded included the type of vegetation found in the area, soil condition (cracked or un-cracked), type of houses and animal shelters.

During the trapping period, a total of 424 sandflies were caught. These sandflies were composed of five *Sergentomyia* (95.76%) and two *Phlebotomus* species (4.24%) (Table 1). At the time of trapping, there was no rainfall and the cracks on the ground were open. Majority of these sandflies (75.23%) were females in all trapping sites. The two *Phlebotomus* species caught were *P. orientalis* (3.77%) and *P. saevus* (0.47%) while the *Sergentomyia* species were *S. schwetzi* Adler, Theodor and Parrot (54.48%), *S. bedfordi* Newstead (1.89%), *S. squamipleuris* Newstead (9.43%), *S. clydei* Sinton (28.54%) and *S. magna* Theodor (1.42%) comprising of the total collection (Table 1).

In most areas of Kenya that have been shown to be endemic for VL, the only phlebotomine sandfly that has been incriminated as the vector is *P. martini*^{5,6}.

Table 1. Percentage number of sandflies collected by species, sexes (M+F) and trap numbers

Sandfly species	Total (Male + Female)	Percentage
P. orientalis	16 (6 + 10)	3.77
P. saevus	2 (0 + 2)	0.47
S. magna	6 (0+6)	1.42
S. bedfordi	8 (1 + 7)	1.89
S. clydei Sinton	121 (14 + 107)	28.54
S. schwetzi Adler	231 (74 + 157)	54.48
S. squamipleuris	40 (10 + 30)	9.43
Total	424 (105 + 319)	100

This sandfly was not trapped within the study area of Merti. The termite mounds, where the vector lives were absent in the two villages. However, considering that P. orientalis is a known vector in the Sudan and that epidemics have been reported to occur in the Merti study area, in the absence of *P. martini*, it is possible that the cause of transmission could be *P*. orientalis. It was reported that P. orientalis is found in Kenya in very small numbers such that this species cannot be a major vector of kala-azar⁸. Our study suggests that this species could be transmitting the disease in the study area in the absence of P. martini. Just like in Sudan where P. orientalis is mainly found, in the study area, Acacia trees and chromic vertisols are the major environmental features¹². The soil cracks that form after the rainy season following flooding become ideal micro-habitats for the breeding and/or resting sites of P. orientalis. It is possible that the species has managed to move southwards from the Sudan into Kenva, where it has managed to thrive well under environmental conditions that favour their existence as was reported earlier⁸. The ground in the study area becomes muddy and waterlogged during the wet season and cracks appear during the drier months¹².

The two villages were selected for this short study due to the large numbers of kala-azar victims exposed during the epidemics⁹. It was our priority to investigate the vector(s) that were transmitting the disease by exposing the identification features like spermatheca, pharynx and cibarial teeth. We did not examine the dissections for Leishmania parasites because the study was intended to show the sandfly species only. Infection rates, parasite identification and blood meal sources will be the scope of our next study. In this study we caught more P. orientalis in Biliqi village than in Ollagana. Interviews with the residents revealed that most cases of kala-azar during the outbreak came from Biliqi village which could be associated with the high numbers of the vector in the village. Ollagana village had fewer cases than Biliqi which could be due to less number of vectors in the village. From these results it is important that, more studies should be carried out to establish the infection rates of the sandfly species involved in the transmission, trap more sandflies, isolate and characterize the *Leishmania* parasites and determine the blood meal sources for *P. orientalis* in the study area.

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